THE DIGESTIVE EPITHELIUM OF DRAGONFLY NYMPHS.

JAMES G. NEEDHAM.

In the aquatic nymphs of dragonflies the alimentary canal fills a *rôle* of unusual importance. It chews, digests, excretes, and respires. More than its name implies, it is in a broad sense an organ of nutrition, or, rather, it is a series of nutritive organs. Its anterior third is concerned with the comminution of the food; its posterior two-fifths with excretion and respiration, and the small remaining middle portion, the ventriculus, alone digests the food, and, according to Cuénot,[1] also alone absorbs it. Recognizing its small size in proportion to the size of the body, and recalling the voracity of these insects, one is prepared to find in it conditions of exceptional activity.

In the dragonflies the ventriculus (*mitteldarm, medi-intestin*) is a simple tube without caeca or diverticula of any sort, slightly

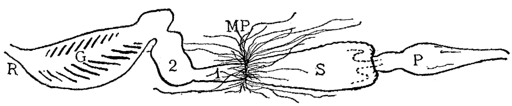

FIG. 1.—Alimentary canal of *Gomphus descriptus* Banks, side view, tracheae removed, × 2. *P*, pre-intestine; *S*, stomach, ventriculus, or mid-intestine; *MP*, Malpighian tubules: 1 and 2 divisions of the post intestine; *G*, gill chamber; *R*, terminal portion of the rectum.

narrowed posteriorly to its junction with the intestine at the origin of the Malpighian tubules. At its anterior end it meets the pre-intestine by a deep circular invagination into which the end of the so-called "gizzard" (*valvule ésophagienne*) descends. Its walls are of nearly uniform structure throughout, consisting of four well-marked layers which are, passing from within outward, (1) a layer of epithelium (not quite a simple layer, as we shall see); (2) a *membrana propria*, or basement membrane; (3) a layer of circular muscles; and (4) an incomplete layer of

[1] CUÉNOT, 1895: Études phys. sur les Orthoptères, *Arch. de Biol.*, xiv.

longitudinal muscle fibers. Sadones[2] has made careful study of all these parts in the nymph of *Libellula depressa* L., and they have been studied in other insects by many investigators.

The part of the ventriculus for which all other parts exist is the epithelium, and although this part has received much attention, there is among investigators little concord of opinion as to the meaning of certain of its structures. The purpose of this paper is to record the results of some additional studies of a combined histological and physiological nature upon this part. Grateful acknowledgment is due Prof. S. H. Gage for kind assistance and advice in the prosecution of these studies.

The nymphs of dragonflies are favorable subjects for studies of this sort, being everywhere common in fresh water, and easily collected, easily kept, and easily fed. A dish of water with some sand and a few plants in it furnished a congenial home for my nymphs so long as I kept them alive; and larvae of *Diptera*, or when these failed, bits of live earthworm, served well for food. The species mostly used were *Leptetrum quadrimaculatum* L., which lives sprawling upon the trashy bottoms of ponds, *Aeschna constricta* Say, which clings to submerged vegetation mostly in streams, and *Gomphus descriptus* Banks, which burrows in the bottom of both streams and ponds. There was found no appreciable difference in structure correlated with their difference of distribution in depth and consequent difference in food; and the species last named was the one used most extensively. It is the one from which the figures have been drawn.

Methods. — For the study of the accumulation of the digestive secretion, some nymphs were kept until wanted in a bare dish of water, where there was no chance of obtaining food. For the study of the discharge of this secretion other nymphs of the same size were fed at regular intervals for a time to bring them into the same condition, and then killed at different intervals after their last feeding. For general morphology, picro-aceto-sublimate (Rath's) was used for fixing, and was fol-

[2] SADONES, 1895: L'Appareil digestif et respiratoire larvaire des Odonates, *La Cellule*, xi.

lowed by paracarmine for staining *in toto*, or by haematoxylin (Gage's) and eosin for staining on the slide. For differentiating functional from nonfunctional parts, Hermann's fluid was used for fixing without subsequent staining. Numerous preparations were made from each of the three species named, at frequent intervals from one to eight hours after feeding. Serial sections of the whole of each preparation were mounted and studied.

I. The Accumulation of the Digestive Secretion.

A well-known type of digestive epithelium in insects consists of more or less elongated cylindric cells which rest upon the *membrana propria* and bear upon their free inner ends a strongly refractive striated border; interspersed among these cylindric cells are little groups or *nidi* of small, roundish cells close to the *membrana propria*, having no communication with the digestive cavity. The cockroach and other *Orthoptera* beautifully exemplify this type. The epithelium of dragonfly nymphs differs from it only by the closer approximation of the *nidi* and the crowding and elongation of the cells between them. Fig. 2 represents the epithelium in its normal resting condition. The digestion of a meal has recently been performed, and the cells have resumed their habitual aspect. The cylindric cells clearly reach the basement membrane. The cells of the nidus are few. The striated border is complete and undulating.[1]

Fig. 3 shows the condition found in a nymph after fasting two weeks. Midway between the nidi the cells have become very much crowded together, their nuclei are flattened as if by pressure, they rise in prominent elevations with very turgid summits from which the striated border has disappeared. The cells of the nidus have also increased in number. An accumulation of granular secretion in the cells between the nidi and their protrusion above the general level are very evident.

I believe that all these cells retain their connection with the basement membrane, although I was unable to trace them to

[1] Compare the single figure by Sadones for *Libellula depressa* L., *loc. cit.*, Pl. II, Fig. 16.

it, or to dissociate them satisfactorily. At *B* in Figs. 3 and 4 are the bases at least of some of them.[1]

Fig. 4 shows the continuation of this process of accumulation as found in a nymph that had been kept in a bare dish of water for two months. I was expecting to find degeneration in the cells of this preparation, but found instead the remarkable condition of things shown in the figure. The cells appeared perfectly healthy. The nidi were full of cells. The striated border had disappeared, or was only faintly discernible in the bottom of the now lumen-like depressions opposite the nidi. The epithelium was about three times its original thickness, and the upper two-thirds of it consisted of the wedge-shaped apices of granular cells crowded above the original level.

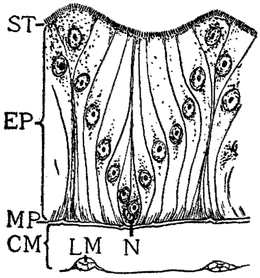

FIG. 2. — Normal resting epithelium soon after the digestion of a meal, × 220. *EP*, the epithelial layer; *ST*, the striated border; *N*, a nidus; *CM*, space filled by the layer of circular muscles; *LM*, longitudinal muscle fibers. Details of the muscles are omitted from all the figures.

Figs. 2, 3, and 4 are from nymphs of the same age and size taken together and kept under identical conditions except as stated above. They are therefore strictly comparable, and together might furnish a morphologist good reasons for taking physiological conditions into account.

II. THE DISCHARGE OF THE DIGESTIVE SECRETION.

After food is eaten it is perhaps an hour before it makes its appearance in the ventriculus. As soon as it enters, the more turgid of the epithelial cells begin to be discharged bodily in whole or in a large part to mix with it. This discharge begins

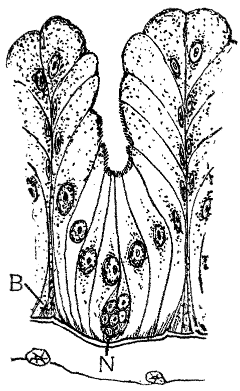

FIG. 3. — Resting epithelium after two weeks' fasting, × 220: parts as in Fig. 2. At *B* are the bases of cells to which the wedge-shaped apices opposite are believed to belong.

[1] There is no supporting connective tissue between these cells. In *Corydalis cornuta* L. the basement membrane is produced upward in anastomosing plates with which all the cells are in evident contact.

at the anterior end and continues posteriorly with the progress of the food. When the nymph has gone long without food and there is much accumulation, the discharge is correspondingly great. It is in fact remarkable to see how large a part of the epithelium will thus at once be destroyed. I have chosen, however, to show in the drawings the discharge taking place after moderate or slight accumulation, as being more normal and on the whole more instructive.

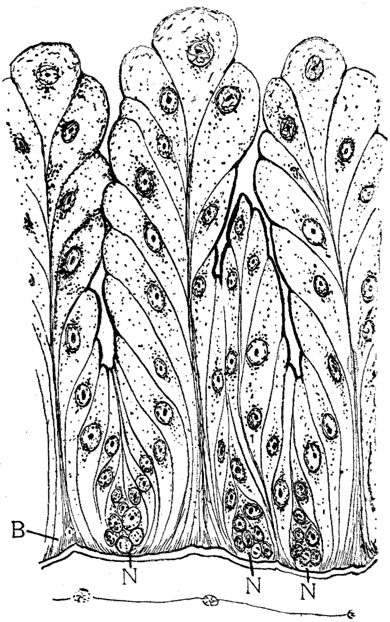

Fig. 4. — Resting epithelium after two months' fasting, × 220: parts as in Figs. 2 and 3.

Fig. 5 represents a normal discharge from epithelium in which the accumulation had been slight and somewhat irregular. The portion included between two nidi on the right side is discharged; that on the left unaffected. The preparation was made from a nymph fed an hour and a half before fixing and from a region just before the middle of the ventriculus.

Each globule of discharge represents the larger part of an epithelial cell, including generally its nucleus. Sometimes the globule appears to be cut off from the cell substance below it by a wall formed previous to its discharge, but much more often it presents the appearance of having been crowded out by the compression of adjacent cells, in which case it is narrowed to a more or less slender point (*B*, Fig. 5). In no case have I seen a small portion of the cell contents protruding through a cleft in the striated border as found by Van Gehuchten[3] in *Ptychoptera contaminata;* but in all cases the discharge involved the whole free end of the discharging cell from which the striated border had disappeared as if by solution.

[3] Van Gehuchten, 1890: Recherches histologiques sur l'appareil digestif de la larve de la Ptychoptera contaminata, *La Cellule*, vi.

Fig. 6 is from a nymph that was fed three hours before fixing. A large area (*D*) is discharged; groups of cells remain in which the discharge is yet to occur.

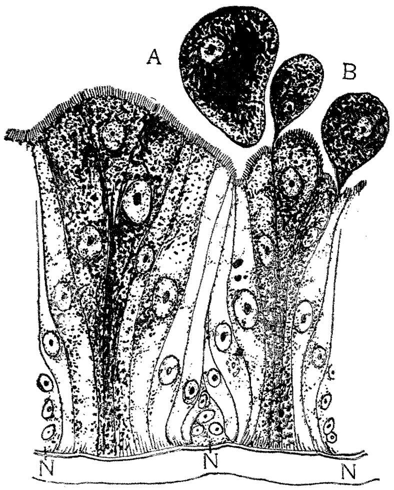

FIG. 5. — The beginning of a discharge in which the left-hand portion shown is not yet involved, two hours after feeding, × 275. *A*, a large detached globule of secretion. *B*, smaller globules still slightly connected by a slender stalk-like portion, compressed between the remaining cells.

Fig. 7 is from the same series, but shows a larger area in which a very moderate discharge is in various stages of progress. The forked summits of the cells at the right seem to indicate that the discharge in them was partial. Two nuclei are found not uncommonly in these cells (see Figs. 4, 6, 8), only one of which appears to be lost at a time.

Fig. 8 is from the same series, but from a point nearer the anterior end, and thus represents a later stage. It shows, in fact, the end of the discharge. The beginning occurred midway between the nidi, but these last rounded globules came from points nearly opposite them. The intervening cells have already acquired a border; they have plenty of room, are now nearly cylindrical, and the prominent internal folds (Figs. 2, 3, 4), due to later compression, have not yet appeared. A study of hundreds of such sections shows the progress of the discharge to be about as follows. The discharge begins in the anterior end of the ventriculus about an hour after feeding simultaneously with

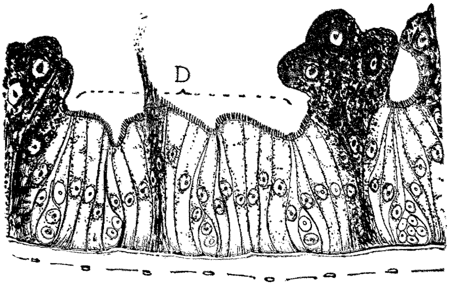

FIG. 6. — An irregular discharge found three hours after feeding, × 150. The discharge is complete throughout the portion *D*, on the point of beginning on either side. These different conditions found side by side afford opportunity for an interesting comparison of the state of the nidi.

the appearance of the food. Here it reaches its height in something less than two hours, and in three hours is about finished. It progresses slowly through the length of the ventriculus, beginning at the posterior end in something less than three hours, and being completed in six to eight hours after feeding. Thus in the posterior end, where the cells are somewhat longer and more numerous, it will be seen the discharge occupies a longer time.

III. The Replacement of the Discharged Cells.

The long cells which extend from the basement membrane to the lumen of the ventriculus and the little round cells nestled together against the basement membrane form two markedly

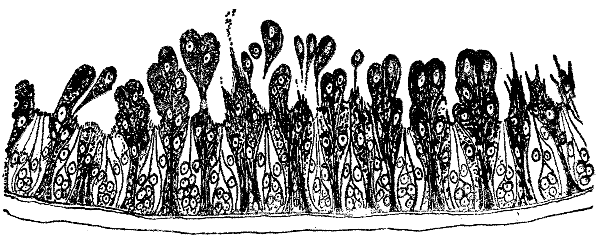

FIG. 7.—A normal discharge of the digestive secretion from the same series as Fig. 6, × 105.

different parts of the epithelium. The latter have been discussed under various names corresponding to the diverse views held as to their function. They have been called *cryptes*, *drüsencrypten*, and *drüsen* by Basch,[4] Frenzel,[5] and Faussek[6] respectively. More recently Visart[7] has considered them to be glands. This view has been copied widely.

They have been called *germinal buds* by Miall and Denny,[8]

[4] BASCH, 1858: Untersuchungen über Chylopoetische und uropoetische System der Blatta orientalis, *Sitzungsbr. der k. k. Akad. Wiss. Wien, math.-nat. Klasse*, xxxiii.

[5] FRENZEL, 1885: Einiges über den Mitteldarm der Insekten sowie über Epithelregeneration, *Archiv. Micr. Anat.*, xxvi.

[6] FAUSSEK, 1887: Beiträge zur Histologie des Darmkanals der Insekten, *Zeit. Wiss. Zool.*, xlv.

[7] VISART, 1894: Contr. &c. tubo digerenti Arthropoda, *Atti d. Soc. Toscano de Sci. Nat.*, xiii. Of this paper I have seen only abstracts.

[8] MIALL and DENNY: The Cockroach, London, 1886.

and have been discussed as centers of replacement by Balbiani,[9] Bizzozzero,[10] and others. Throughout my work I have used the term *nidus* as a convenient, descriptive, noncommittal name.

Most of the recent investigators have looked upon them as centers of regeneration, with various reasons for taking that view. Van Gehuchten, finding the nucleus to be discharged with the secretion, based his belief largely on the theory that when a cell loses its nucleus it must die and be replaced. Balbiani found nidi made up of cells varying in number and size, and assumed these to represent different stages of development. Bizzozzero and Cuénot attach most significance to the mitotic figures found in the nidi and not found elsewhere in the epithelium, a fact noted by many writers. This evidence is good so far as it goes. The mitotic figures indicate that cell division is in progress here, but tell nothing of what may be doing elsewhere. It is now my privilege to add as a result of experimental studies some additional evidence of the correctness of the view that each nidus is a germinative center.

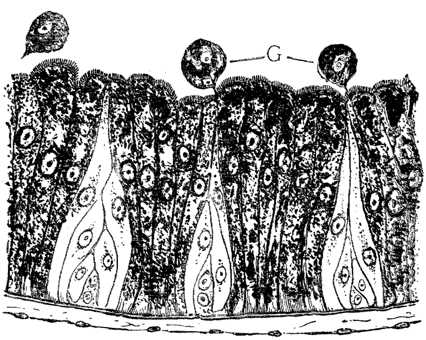

FIG. 8. — The end of the discharge, × 220, from the same series as Figs. 6 and 7, but from the anterior end of the ventriculus. *G*, the last of the globules to be thrown off; these are nearly opposite the nidi.

This evidence is based on observations upon (1) the *source* of the *digestive secretion;* (2) *differentiation by reagents;* (3) the *position* of the nuclei; (4) the decrease in the number of cells in the nidi during digestion; and (5) the encroachment of young cells upon the old.

(1) After tracing the discharge from beginning to end through several series of experiments, it is perfectly evident that the

[9] BALBIANI, 1890: Études anatomiques et histologiques sur le tube digestif des Cryptops, *Arch. de Zool. Exp.*, xi.

[10] BIZZOZZERO, 1893: Ueber die Schlauchförmigen Drüsen, etc., *Arch. Micr. Anat.*, xlii.

digestive secretion is given off from the cells between the nidi, and never any part of it from the nidi themselves. This is as shown in preceding figures. The slender tube Frenzel found connecting the nidus with the digestive cavity (which made it desirable for the *cryptes* of Basch to be rechristened *drüsen crypten*) has no existence. What so appeared was doubtless the slender prolongation of a young cell growing toward the ental surface.

(2) After witnessing the discharge of the digestive secretion, there is no question as to the part that is functional in producing it; and when that part is affected by a given reagent while another is not, the second must be of different character. Hermann's fluid blackens all those epithelial cells which are discharging or ready to discharge their secretion, and leaves the small cells of the nidus, the membrana propria, and muscles equally pale and clear. With haematoxylin and eosin, the eosin stains deeply the functional cells, including the nuclei of the more elevated ones, while the haematoxylin with its usual selectivity for germinative protoplasm stains the cells of the nidus most deeply.

(3) In sections favorable for showing the typical structure the nuclei of the cells are arranged in rows extending from the nidus obliquely upward into the folds (see Figs. 2–4); these lines indicate the course of their progress toward the surface. I have whole series of sections of the stage shown in Fig. 3, throughout which the nuclei are beautifully festooned between the elevations of the ental surface. Naturally, the nidus shows as in the figure only when the section is central. An irregularity of common occurrence is shown at the right side of Fig. 4, while the left side of that figure is quite regular. In nearly every case it is easy to follow two outgoing lines of nuclei from each nidus.

(4) When the accumulation of digestive secretion is considerable, the nuclei of the nidus are numerous; after its discharge one finds but few of them remaining. But if one will add to the number remaining the number of the new cells which have suddenly appeared beside them which are not blackened by Hermann's fluid, he will have a number about equal to the

original number of nuclei in the nidus, a fact sufficiently accounting for their disappearance. Within the nidus at the right of Fig. 6, the cells are more numerous than in those nidi within the area from which the secretion has entirely been given off. Compare also Figs. 3 and 8.

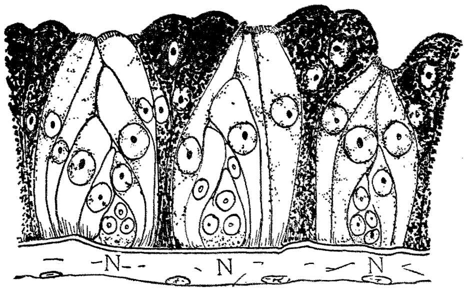

FIG. 9. — Epithelium, six hours after feeding, showing newly formed cells reaching up to the surface, replacing those discharged, × 220.

(5) So far as one may judge, division within the nidus takes place with great regularity. As new cells develop at the base of the nidus, the older ones are crowded upward. The nucleus of each grows rapidly, and soon acquires a spindle-shaped cell body. When preëxisting cells are not being used up in the digesting of food, the young ones thus formed grow slowly, and making their way upward against the compression of the older cells, are crowded into crescentic form. But they continue to increase, nevertheless, as a glance at Figs. 2, 3, and 4 shows. But when the presence of food occasions the removal of the surcharged older cells, the increase in size of the younger ones is remarkably rapid. Just before a discharge, Hermann's fluid blackens all the cells except the spherical ones immediately within the nidus: just after, it leaves several cells on each side entirely clear: and the inference is that these cells have sprung up during that short interval, the lateral compression of the older cells being removed by their discharge, and have attained their growth but have not yet become functional.

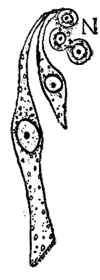

FIG. 10. — A dissociation, showing the constitution of the nidus *N*, × 240.

Fig. 9 shows the young cells apparently in the act of crowding their way to the surface. The blackened area represents all that remains of the part that was functional before the last discharge. The clear area consists of cells that were uppermost in the nidus, but which have suddenly grown up to adult and functional proportions. If this

be not actual replacement happening before one's eyes, it comes as near it as the nature of the case will admit.

One naturally asks what becomes of the remnants of the old cells, and whether there is any connection between their disappearance and the rapid growth of the young cells. On these questions I have obtained no light.

Summarizing their history, we may say, then, in a word, the epithelial cells originate by divisions in the cells of the nidus, grow, crowd their way to the surface, acquire a striated border, become functional, secrete, discharge, digest, and die, and then give place to others which will run the same swift course.